

Patent claims:

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1. Process for the amplification and quantitative real-time detection of nucleic acids, characterized in that
 - a) a primer is used to which a nucleic acid sequence, preferably with a length of 1 to 40 nucleotides, is attached, which codes for the sequence motif 5'-GAAA-3' (motif A) in the transcript,
 - b) the amplification being carried out in the presence of an excess, preferably in a concentration of 50 to 500 nM, of a nucleic acid probe, preferably with a length of 25 to 60 nucleotides (particularly preferably approx. 50 nucleotides) which contains the sequence motif 5'-CUGANGA-3' (motif B), a reporter molecule and a quencher molecule being attached to each probe molecule, and
 - c) the original concentration of the nucleic acid in the sample is determined by measuring the time-dependent change in fluorescence during amplification, the relative concentration " $C_{rel.}$ "

being determined according to the following formula:

$$C_{rel.} = t_i / t_{Ref.}$$

where

t_p corresponds to the time measured for the sample from the start of amplification to the reaching of the fluorescence threshold value and

$t_{Ref.}$ corresponds to time measured for a reference nucleic acid of known concentration from the start of amplification to the reaching of the fluorescence threshold value.

2. Process for the amplification and quantitative real-time detection of nucleic acids, characterized in that

- a) a primer is used to which a nucleic acid sequence, preferably with a length of 1 to 40 nucleotides, is attached, which codes for the sequence motif 5'-CUGANGA-3' (motif B) in the transcript,
- b) the amplification is carried out in the presence of an excess, preferably in a concentration of 50 to 500 nM, of a nucleic acid probe, preferably with a length of 25 to 60 nucleotides (particularly preferably approx. 50 nucleotides) which contains the sequence motif 5'-GAAA-3' (motif A), a reporter molecule and a quencher molecule being attached to each probe molecule, and
- c) the original concentration of the nucleic acid in the sample is determined by measuring the time-dependent change in fluorescence during the amplification, the relative concentration " $C_{rel.}$ " being determined according to the following formula:

$$C_{rel.} = t_p / t_{Ref.}$$

where

t_p corresponds to the time measured for the sample from the start of the amplification to the reaching of the fluorescence threshold value and

$t_{ref.}$ corresponds to time measured for a reference nucleic acid of known concentration from the start of the amplification to the reaching of the fluorescence threshold value.

3. Process for the amplification and quantitative real-time detection of a nucleic acid containing the sequence motif 5'-GAAA-3' (motif A), characterized in that

- a) the sequences of the primers used are chosen such that the sequence range of the nucleic acid which contains motif A is amplified,
- b) the amplification being carried out in the presence of an excess of a nucleic acid probe which contains the sequence motif 5'-CUGANGA-3' (motif B), a reporter molecule and a quencher molecule being attached to each probe molecule, and
- c) the original concentration of the nucleic acid in the sample is determined by measuring the time-dependent change in fluorescence during the amplification, the relative concentration " $C_{rel.}$ " being determined according to the following formula:

$$C_{rel.} = t_p / t_{Ref.}$$

where

t_p corresponds to the time measured for the sample from the start of the amplification to the reaching of the fluorescence threshold value and

$t_{Ref.}$ corresponds to the time measured for a reference nucleic acid of known concentration from the start of the amplification to the reaching of the fluorescence threshold value.

4. Process for the amplification and quantitative detection of a nucleic acid containing the sequence motif 5'-CUGANGA-3' (motif B), characterized in that
- a) the sequences of the primers used are chosen such that the sequence range of the nucleic acid which contains motif B is amplified,
 - b) the amplification being carried out in the presence of an excess of a nucleic acid probe which contains the sequence motif 5'-GAAA-3' (motif A), a reporter molecule and a quencher molecule being attached to each probe molecule, and
 - c) the original concentration of the nucleic acid in the sample is determined by measuring the time-dependent change in fluorescence during the amplification, the relative concentration " C_{rel} ." being determined according to the following formula:

$$C_{rel} = t_p / t_{ref.}$$

where

t_p corresponds to the time measured for the sample from the start of the amplification to the reaching of the fluorescence threshold value and

$t_{ref.}$ corresponds to the time measured for a reference RNA of known concentration from the start of the amplification to the reaching of the fluorescence threshold value.

- 5. Process according to claim 1, characterized in that the nucleic acid is RNA, DNA or a DNA/RNA chimera.
- 6. Process according to claim 1, characterized in that the nucleic acid sequence attached to the primer has a length of 1 to 40 nucleotides.
- 7. Process according to claim 1, characterized in that the nucleic acid probe is used in a concentration of 50 to 500 nM.

8. Process according to claim 1, characterized in that the nucleic acid probe has a length of 25 to 60 nucleotides, preferably approx. 50 nucleotides.
9. Process according to claim 1, characterized in that the amplification process is an isothermal or cyclical amplification process.
10. Process according to claim 9, characterized in that the amplification process is selected from the group consisting of NASBA[®], TMA, 3SR or PCR.
11. Process according to claim 1, characterized in that there is used, as reporter, a dye from the group consisting of FAM, HEX, TET, ALEXA, Texas Red, Light Cycler Red, IRD 700, CY-7, IRD 41 or La Jolla Blue and, as quencher, a dye from the group consisting of TAMRA, CY-5, DABCYL and LCR.
12. Process for the detection of bacterial pathogens in a sample, characterized in that the sample is brought into contact with a probe which contains the sequence motif 5'-CUGANGA-3' (motif B), a reporter molecule and a quencher molecule being attached to each sequence motif, the probe containing a sequence suitable for the hybridization with a section of the 16S rRNA of the pathogens containing the sequence motif 5'-GAAA-3' (motif A), and the pathogens being detected by measuring the occurring fluorescence signal.
13. Process according to claim 12, characterized in that the pathogens are selected from the group consisting of E. coli, Salmonella, Staphylococcus, C. perfringens, Vibrio, B. cereus, C. botulinum, Campylobacter, Yersinia and Listeria.
14. Process according to claim 12, characterized in that the nucleic acid probe has a length of 25 to 60 nucleotides, preferably approx. 50 nucleotides.
15. Process according to claim 12, characterized in that there is used as reporter, a dye from the group consisting of FAM, HEX, TET, ALEXA, Texas Red, Light Cycler Red, IRD 700, CY-

7, IRD 41 or La Jolla Blue and, as quencher, a dye from the group consisting of TAMRA, CY-5, DABCYL and LCR.

16. Kit for carrying out the process according to claim 1, characterized in that it comprises
- a) an amplification primer to which a nucleic acid sequence is attached, which codes for the sequence motif 5'-GAAA-3' in the transcript,
 - b) a further amplification primer,
 - c) enzymes and reagents for carrying out the amplification,
 - d) a nucleic acid probe which contains the sequence motif 5'-CUGANGA-3', a reporter molecule and a quencher molecule being attached to each probe molecule, as well as optionally
 - e) apparatus and auxiliaries necessary for carrying out the reaction.
17. Kit for carrying out the process according to claim 2, characterized in that it comprises
- a) an amplification primer to which a nucleic acid sequence is attached, which codes for the sequence motif 5'-CUGANGA-3' in the transcript,
 - b) a further amplification primer,
 - c) enzymes and reagents for carrying out the amplification,
 - d) a nucleic acid probe which contains the sequence motif 5'-GAAA-3', a reporter molecule and a quencher molecule being attached to each probe molecule, as well as optionally
 - e) apparatus and auxiliaries necessary for carrying out the reaction.
18. Kit for carrying out the process according to claim 3, characterized in that it comprises
- a) two amplification primers,
 - b) enzymes for carrying out the amplification,
 - c) a nucleic acid probe which contains the sequence motif 5'-CUGANGA-3', a reporter molecule and a quencher

molecule being attached to each probe molecule, as well as optionally

- d) apparatus and auxiliaries necessary for carrying out the reaction.
19. Kit for carrying out the process according to claim 4, characterized in that it comprises
- a) two amplification primers,
 - b) enzymes for carrying out the amplification,
 - c) a nucleic acid probe which contains the sequence motif 5'-GAAA-3', a reporter molecule and a quencher molecule being attached to each probe molecule, as well as optionally
 - d) apparatus and auxiliaries necessary for carrying out the reaction.
20. Kit according to claim 16, characterized in that the nucleic acid is RNA, DNA or a DNA/RNA chimera.
21. Kit according to claim 16, characterized in that the nucleic acid sequence attached to the primer has a length of 1 to 40 nucleotides.
22. Kit according to claim 16, characterized in that the nucleic acid probe is used in a concentration of 50 to 500 nM.
23. Kit according to claim 16, characterized in that the nucleic acid probe has a length of 25 to 60 nucleotides, preferably approx. 50 nucleotides.
24. Kit according to claim 16, characterized in that the amplification process is an isothermal or cyclical amplification process.
25. Kit according to claim 24, characterized in that the amplification process is selected from the group consisting of NASBA[®], TMA, 3SR or PCR.

26. Kit according to claim 25, characterized in that it is a kit for carrying out a NASBA[®], the enzymes displaying the activity of reverse transcriptase, T7 RNA polymerase and RNase H.
27. Kit according to claim 26, characterized in that the enzymes for carrying out the NASBA[®] are reverse transcriptase, T7 RNA polymerase and RNase H.
28. Kit for carrying out the process according to one of claim 12, characterized in that it comprises a probe with a sequence suitable for the hybridization with a section of the 16S rRNA of the pathogens containing the sequence motif 5'-GAAA-3' (motif A), which contains the sequence motif 5'-CUGANGA-3' (motif B), a reporter molecule and a quencher molecule being attached to each probe molecule, as well as optionally further apparatus and auxiliaries necessary for carrying out the reaction.
29. Kit according to claim 16, characterized in that the reporter is a dye from the group consisting of FAM, HEX, TET, ALEXA, Texas Red, Light Cycler Red, IRD 700, CY-7, IRD 41 or La Jolla Blue and the quencher a dye from the group consisting of TAMRA, CY-5, DABCYL, and LCR.

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